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sequence_AA_2D12.5_variable domains.txt

```
>2D12.5VL_MOUSE
(1) QAVVTQESALTTSPGETVTLICRSSTGAVTTSNYANWVQEKPDHLFTGLIGNNNRPPGVPARFSGSLIGDKAALTIAGTQTED
    EAIYFCALWYSNHWVFGGGTRLTVLG

(2) CDR1 - RSSTGAVTTSNYAN
(3) CDR2 - GNNNRPP
(4) CDR3 - ALWYSNHWV

>2D12.5VH_MOUSE
(5) QVKLQESGPGLVQPSQSLSTCTVSGFSLTDYGVHWVRQSPGKGLEWLGVIWSGGGTAYTAAAFISRLNIY
    KDNSKNQVFFEMNSLOANDTAMYYCARRGSYPYNYFDVWGQGTTVTVSS

(6) CDR1 - DYGVH
(7) CDR2 - VIWSGGGTAYTAAAFIS
(8) CDR3 - RGSYPYNYFDV
```

FIG. 1

Translation of 2d12.5 VH variable genes

(9)2d12.5 VH native hybridoma	1	VKLQESGPGLVQPSQSLSITCTVSGFSLTDYGVHVV RQSPGKGLEWLGVI	50
(10)2d12.5 VH native cloned	1	50
(11)2d12.5 VH N87D_cloned	1	50
(12)2d12.5 VH N87D_G53C_cloned	1	50
(13)2d12.5 VH N87D_G54C_cloned	1	50
(14)2d12.5 VH N87D_G55C_cloned	1	50

2d12.5 VH native hybridoma	51	WSGGGTAYTAAFISRLNIYKDNSKNQVFFEMNSLQANDTAMYCARRGSY	100
2d12.5 VH native cloned	51	100
2d12.5 VH N87D_cloned	51D.....	100
2d12.5 VH N87D_G53C_cloned	51	...C.....D.....	100
2d12.5 VH N87D_G54C_cloned	51	...C.....D.....	100
2d12.5 VH N87D_G55C_cloned	51	...C.....D.....	100

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2d12.5 VH native hybridoma	101	PYNYFDVWGQGTTVTVSS	118
2d12.5 VH native cloned	101A	118
2d12.5 VH N87D_cloned	101A	118
2d12.5 VH N87D_G53C_cloned	101A	118
2d12.5 VH N87D_G54C_cloned	101A	118
2d12.5 VH N87D_G55C_cloned	101A	118

FIG. 2

2D12.5 VH variable genes

(15) 2d12.5 VH native hybridoma	1	GTGAAGCTGCAGGAGTCAGGACCTGGCCCTAGTCAGCCCTCAGAGCCT	50
(16) 2d12.5 VH native cloned	1T.....	50
(17) 2d12.5 VH N87D cloned	1T.....	50
(18) 2d12.5 VH N87D_G53C cloned	1T.....	50
(19) 2d12.5 VH N87D_G54C cloned	1T.G.....	50
(20) 2d12.5 VH N87D_G55C cloned	1T.....	50

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2d12.5 VH native hybridoma	51	GTCCATCACCTGCACGGTCTCTGGTTTCTCATTAAGTACTATGGTGTAC	100
2d12.5 VH native cloned	51	100
2d12.5 VH N87D cloned	51	100
2d12.5 VH N87D_G53C cloned	51	100
2d12.5 VH N87D_G54C cloned	51	100
2d12.5 VH N87D_G55C cloned	51	100

2d12.5 VH native hybridoma	101	ACTGGGTTCCGCAGTCTCCAGGAAGGGTCTGGAATGGCTGGGAGTGATA	150
2d12.5 VH native cloned	101	150
2d12.5 VH N87D cloned	101	150
2d12.5 VH N87D_G53C cloned	101	150
2d12.5 VH N87D_G54C cloned	101	150
2d12.5 VH N87D_G55C cloned	101	150

FIG. 3A

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FIG. 3A (CONT.)

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	310	320	330	340	350
				
2d12.5 VH native hybridoma	301 CCTTAACTACTTCGATGTC	320 TGGGGCCAAAGGACCA	330 GACAGTCACCGTCTC	340	350
2d12.5 VH native cloned	301	320	330	340 G.....	350
2d12.5 VH N87D cloned	301	320	330	340 G.....	350
2d12.5 VH N87D_G53C cloned	301	320	330	340 G.....	350
2d12.5 VH N87D_G54C cloned	301	320	330	340 G.....	350
2d12.5 VH N87D_G55C cloned	301	320	330	340 G.....	350

FIG. 3A (CONT.)

2D12.5 VH variable genes

2d12.5 VH native hybridoma	351	CTCA	354
2d12.5 VH native cloned	351	.G..	354
2d12.5 VH N87D cloned	351	.G..	354
2d12.5 VH N87D_G53C cloned	351	.G..	354
2d12.5 VH N87D_G54C cloned	351	.G..	354
2d12.5 VH N87D_G55C cloned	351	.G..	354

FIG. 3B

Translation of 2D12.5 VL genes

		10	20	30	40	50	
						
(21)2d12.5 VL native hybridoma	1	AVVTQESALTTSPGETVTLTCRSSSTGAVTTSNYANWVQEKPDHLFTGLIG	50				
(22)2d12.5 VL native cloned	1	50				
(23)2d12.5 VL N53C_cloned	1	50				
		60	70	80	90	100	
						
2d12.5 VL native hybridoma	51	GNNRPPGVPARFSGSLIGDKAALTIAGTQTEDEAIYFCALWYSNHWVFG	100				6/41
2d12.5 VL native cloned	51	100				
2d12.5 VL N53C_cloned	51	.C.....	100				
						
2d12.5 VL native hybridoma	101	GGTRLTVLG	109				
2d12.5 VL native cloned	101	...K....S	109				
2d12.5 VL N53C_cloned	101	...K....S	109				

FIG. 4

FIG. 5

FIG. 5 (CONT.)

Translation of Mouse 2D12.5 VL - Human TetTox CL kappa (light chain gene)

	10	20	30	40	50																		
(27) 2dVL-TTCL native_cloned	1	RS	AV	TQ	ES	AL	TT	SP	GE	TV	TL	TC	RS	SS	GA	VT	TS	NY	AN	WV	QEKPDHLFTGL	50
(28) 2dVL-TTCL N53C_cloned	1	50
(29) 2d12.5 VL native hybridoma	1	--	50
(30) TTCL template for gene assembly	1	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

	60	70	80	90	100																							
2dVL-TTCL native_cloned	51	IG	NN	NR	PP	GP	PA	RF	SG	SL	IG	DK	AA	LT	IA	GT	EQ	TE	DE	AI	YF	CA	LW	YS	NH	WV	100
2dVL-TTCL N53C_cloned	51	100	
2d12.5 VL native hybridoma	49	98	
TTCL template for gene assembly	1	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	

	110	120	130	140	150																						
2dVL-TTCL native_cloned	101	FG	GT	KL	TV	LS	RT	VA	AP	SV	FI	FP	PS	DE	QK	SG	TA	SV	VV	CL	LN	NN	FY	PR	EAKV	150
2dVL-TTCL N53C_cloned	101	150
2d12.5 VL native hybridoma	99	100
TTCL template for gene assembly	1	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	80

FIG. 6

2dVL-TTCL native_cloned	151 QWKVDNALQSGNSQESVTEQDDSKDSTYSLSSTLTLSKADYEKHKVYACEV	200
2dVL-TTCL N53C_cloned	151	200
2d12.5 VL native hybridoma		
TTCL template for gene assembly	40	80
2dVL-TTCL native_cloned	201 THQGLSLPVTKSFNRGEC*F*221	220
2dVL-TTCL N53C_cloned	201	220
2d12.5 VL native hybridoma		
TTCL template for gene assembly	90	107

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FIG. 6 (CONT.)

FIG. 7A

Mouse 2D12.5 VL - Human TetTox CL kappa (light chain gene)

	10	20	30	40	50
				
(31)2dVL-TTCL native_cloned	1				50
(32)2dVL-TTCL N53C_cloned	1				50
(33)2d12.5 VL native hybridoma	1				44
(34)TTCL template for gene assem	1				1
	60	70	80	90	100
				
2dVL-TTCL native_cloned	51				100
2dVL-TTCL N53C_cloned	51				100
2d12.5 VL native hybridoma	45				94
TTCL template for gene assem	1				1
	110	120	130	140	150
				
2dVL-TTCL native_cloned	101				150
2dVL-TTCL N53C_cloned	101				150
2d12.5 VL native hybridoma	95				144
TTCL template for gene assem	1				1
	160	170	180	190	200
				
2dVL-TTCL native_cloned	151				200
2dVL-TTCL N53C_cloned	151				200
2d12.5 VL native hybridoma	145				194
TTCL template for gene assem	1				1

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```

210      220      230      240      250
...|...|...|...|...|...|...|...|...|...|
201 CTCCCTGATTGGAGACAAGGCTGCCCTCACCATCGCAGGGACACAGACTG 250
201      250
...|...|...|...|...|...|...|...|...|...|
195      244
...|...|...|...|...|...|...|...|...|...|
TTCL template for gene assem 1

260      270      280      290      300
...|...|...|...|...|...|...|...|...|...|
251 AGGATGAGGCAATATATTCTGTGCTCTATGGTACAGCAACCATTGGGTG 300
251      300
...|...|...|...|...|...|...|...|...|...|
245      294
...|...|...|...|...|...|...|...|...|...|
TTCL template for gene assem 1

310      320      330      340      350
...|...|...|...|...|...|...|...|...|...|
301 TTCGGTGGGGAACCAAACTGACTGTCTCTAAGCCGAACTGTGGCTGCACC 350
301      350
...|...|...|...|...|...|...|...|...|...|
295      327
...|...|...|...|...|...|...|...|...|...|
TTCL template for gene assem 1

360      370      380      390      400
...|...|...|...|...|...|...|...|...|...|
351 ATCTGTCTTCATCTTCCCGCCCATCTGATGAGCAGTTGAAATCTGGAAC TG 400
351      400
...|...|...|...|...|...|...|...|...|...|
TTCL template for gene assem 18
```

FIG. 7A (CONT.)

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2dVL-TTCL native_cloned	401	410	420	430	440	450
2dVL-TTCL N53C_cloned	401	410	420	430	440	450
2d12.5 VL native hybridoma	401	410	420	430	440	450
TTCL template for gene assem	68	410	420	430	440	117

FIG. 7A (CONT.)

2dVL-TTCL native_cloned	460	470	480	490	500
				
451 CAGTGG AAGTGG ATAA CGCCCTCC AATCGGGTAA CTCCAGGAGAGTGT					500
2dVL-TTCL N53C_cloned					
451					500
2d12.5 VL native hybridoma					
118					167
TTCL template for gene assem					
	510	520	530	540	550
				
501 CACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCCTGA					550
2dVL-TTCL native_cloned					
501					550
2d12.5 VL native hybridoma					
168					217
TTCL template for gene assem					
	560	570	580	590	600
				
551 CGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCCTGCGAAGTC					600
2dVL-TTCL native_cloned					
551					600
2d12.5 VL native hybridoma					
218					267
TTCL template for gene assem					

FIG. 7B

	610	620	630	640	650	
					
2dVL-TTCL native_cloned	601	ACCCATCAGGGCCTGAGCTTGCCCGTCACAAAGAGCTTCAACAGGGGAGA	650			
2dVL-TTCL N53C_cloned	601T.....	650			
2d12.5 VL native hybridoma						
TTCL template for gene assem	268	317			
	660					
					
2dVL-TTCL native_cloned	651	GTGTTAATTCTAGA	664			
2dVL-TTCL N53C_cloned	651	664			
2d12.5 VL native hybridoma						
TTCL template for gene assem	318	322			

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FIG. 7B (CONT.)

	110	120	130	140	150
.....					
101 SYPNYFDVWGQTTVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVK					
2dVH-TTCH_native cloned					150
101					
2dVH-TTCH_N87D_cloned					150
101					
2dVH-TTCH_N87D_G53C_cloned					150
101					
2dVH-TTCH_N87D_G54C_cloned					150
101					
2dVH-TTCH_N87D_G55C_cloned					150
101					
2dVH-TTCH expected sequence					150
101					
2d12.5 VH native hybridoma		S			118
99					

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2dVH-TTCH native cloned	151	DYFPEPVT	160	170	180	190	200
2dVH-TTCH_N87D_cloned	151	TVSWNSGALT	160	170	180	190	200
2dVH-TTCH_N87D_G53C_cloned	151	TVSWNSGALT	160	170	180	190	200
2dVH-TTCH_N87D_G54C_cloned	151	TVSWNSGALT	160	170	180	190	200
2dVH-TTCH_N87D_G55C_cloned	151	TVSWNSGALT	160	170	180	190	200
2dVH-TTCH expected sequence	151	TVSWNSGALT	160	170	180	190	200
2d12.5 VH native hybridoma	151	TVSWNSGALT	160	170	180	190	200

2dVH-TTCH native cloned	201	YICNVN	210	220	227
2dVH-TTCH_N87D_cloned	201	YICNVN	210	220	227
2dVH-TTCH_N87D_G53C_cloned	201	YICNVN	210	220	227
2dVH-TTCH_N87D_G54C_cloned	201	YICNVN	210	220	227
2dVH-TTCH_N87D_G55C_cloned	201	YICNVN	210	220	227
2dVH-TTCH expected sequence	201	YICNVN	210	220	227
2d12.5 VH native hybridoma	201	YICNVN	210	220	227

FIG. 8 (CONT.)

Mouse 2D12.5 VH - Human TetTox CH1 (heavy chain Fab gene)

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2dVH-TTCH_native cloned	151	GTGATATGGAGTGGTGGAGGCACGGCCCTATACTGCGGGTTTCATATCCAG	200
2dVH-TTCH_N87D_cloned	151	200
2dVH-TTCH_N87D_G53C_cloned	151	200
2dVH-TTCH_N87D_G54C_cloned	151	200
2dVH-TTCH_N87D_G55C_cloned	151	200
2dVH-TTCH expected sequence	151	200
2d12.5 VH native hybridoma	145	194
2dVH-TTCH_native cloned	201	ACTGAACATCTACAAGGACAATTCCAAGAACCAAGTTTCTTTGAAATGA	250
2dVH-TTCH_N87D_cloned	201	250
2dVH-TTCH_N87D_G53C_cloned	201	250
2dVH-TTCH_N87D_G54C_cloned	201	250
2dVH-TTCH_N87D_G55C_cloned	201	250
2dVH-TTCH expected sequence	201	250
2d12.5 VH native hybridoma	195	244
2dVH-TTCH_native cloned	251	ACAGTCTGCAAGCTAATGACACAGCCATGTATTACTGTGCCAGAAGGGGT	300
2dVH-TTCH_N87D_cloned	251	300
2dVH-TTCH_N87D_G53C_cloned	251	300
2dVH-TTCH_N87D_G54C_cloned	251	300
2dVH-TTCH_N87D_G55C_cloned	251	300
2dVH-TTCH expected sequence	251	300
2d12.5 VH native hybridoma	245	294

.....	310	320	330	340	350
---	-----	---	-----	---	-----	---	-----	---	-----

FIG. 9A (CONT.)

Mouse 2D12.5 VH - Human TetTox CH1 (heavy chain Fab gene)

2dVH-TTCH native cloned 301 AGCTACCCTTACAACCTACTTCGATGTCTGGGGCCAAAGGACCACGGTCAC 350
2dVH-TTCH_N87D_cloned 301 350
2dVH-TTCH_N87D_G53C_cloned 301 350
2dVH-TTCH_N87D_G54C_cloned 301 350
2dVH-TTCH_N87D_G55C_cloned 301 350
2dVH-TTCH expected sequence 301 350
2d12.5 VH native hybridoma 295A..... 344

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2dVH-TTCH native cloned 351 400
2dVH-TTCH_N87D_cloned 351 400
2dVH-TTCH_N87D_G53C_cloned 351 400
2dVH-TTCH_N87D_G54C_cloned 351 400
2dVH-TTCH_N87D_G55C_cloned 351 400
2dVH-TTCH expected sequence 351 400
2d12.5 VH native hybridoma 345T... 354

2dVH-TTCH native cloned 401 CCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCCTGGTCAAG 450
2dVH-TTCH_N87D_cloned 401 450
2dVH-TTCH_N87D_G53C_cloned 401 450
2dVH-TTCH_N87D_G54C_cloned 401 450
2dVH-TTCH_N87D_G55C_cloned 401 450
2dVH-TTCH expected sequence 401 450
2d12.5 VH native hybridoma 401 450

FIG. 9B

FIG. 9B (CONT.)

2dVH-TTCH_native cloned
2dVH-TTCH_N87D_cloned
2dVH-TTCH_N87D_G53C_cloned
2dVH-TTCH_N87D_G54C_cloned
2dVH-TTCH_N87D_G55C_cloned
2dVH-TTCH expected sequence
2d12.5 VH native hybridoma

451 460 470 480 490 500
GACTACTTCCCGAACC GG TGACGGTGCTTGGAACTCAGGCGCCCTGAC 500
451 500
451 500
451 500
451 500
451 500
451 G 500

2dVH-TTCH_native cloned
2dVH-TTCH_N87D_cloned
2dVH-TTCH_N87D_G53C_cloned
2dVH-TTCH_N87D_G54C_cloned
2dVH-TTCH_N87D_G55C_cloned
2dVH-TTCH expected sequence
2d12.5 VH native hybridoma

501 510 520 530 540 550
CAGCGGGGTGCACACCTTCCCGGCTGCTCCTACAGTCCCTCAGGACTCTACT 550
501 550
501 550
501 550
501 550
501 550
501 550
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2dVH-TTCH_native cloned
2dVH-TTCH_N87D_cloned
2dVH-TTCH_N87D_G53C_cloned
2dVH-TTCH_N87D_G54C_cloned
2dVH-TTCH_N87D_G55C_cloned
2dVH-TTCH expected sequence
2d12.5 VH native hybridoma

551 560 570 580 590 600
CCCTCAGCAGCGGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACC 600
551 600
551 600
551 600
551 600
551 600
551 600

2dVH-TTCH_native cloned
2dVH-TTCH_N87D_cloned

601 610 620 630 640 650
TACATCTGCAACGCTGAATCACAAGCCAGCAACACCAAGGTGGACAAGAA 650
601 650

Mouse 2D12.5 VH - Human TetTox CH1 (heavy chain Fab gene)					
2dVH-TTCH N87D_G53C_cloned	601	660	670	680
2dVH-TTCH N87D_G54C_cloned	601	660	670	680
2dVH-TTCH N87D_G55C_cloned	601	660	670	680
2dVH-TTCH expected sequence	601	660	670	680
2d12.5 VH native hybridoma					
2dVH-TTCH native cloned	651	660	670	680
2dVH-TTCH N87D_G53C_cloned	651	660	670	680
2dVH-TTCH N87D_G54C_cloned	651	660	670	680
2dVH-TTCH N87D_G55C_cloned	651	660	670	680
2dVH-TTCH expected sequence	651	660	670	680
2d12.5 VH native hybridoma					

FIG. 9C

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Strategy for Assembly of Chimeric 2D12.5
 Heavy Chain
 Step 1

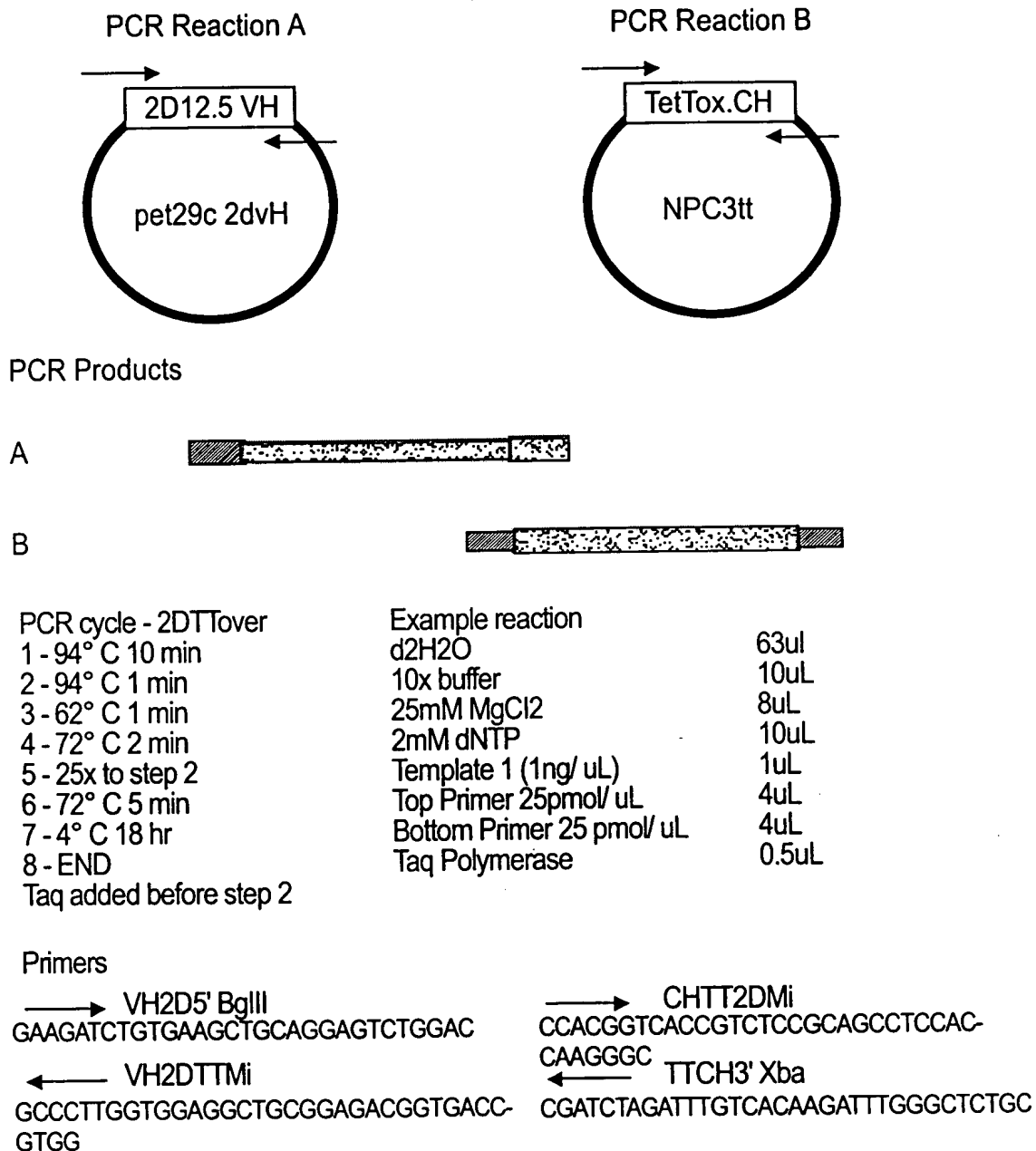
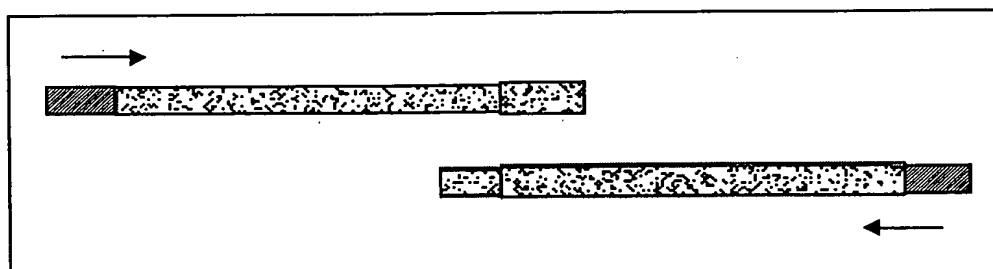


FIG. 10A

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Strategy for Assembly of Chimeric 2D12.5
 Heavy Chain
 Step 2

PCR Reaction



PCR cycle - 2DTTVent

- 1 - 95°C 10 min
 - 2 - 94°C 1 min
 - 3 - 60°C 1 min
 - 4 - 75°C 2 min
 - 5 - 4x to step 2
 - 6 - 94°C 1 min
 - 7 - 63°C 1 min
 - 8 - 75°C 2 min
 - 9 - 25x to step 6
 - 10 - 72°C 5 min
 - 11 - 4°C 18 hr
 - 12 - END
- Vent added before step 2
 Primers added before step 6

Primers

→ VH2D5' BglII
 GAAGATCTGTGAAGCTGCAGGAGTCTGGACC

← TTCH3' Xba
 CGATCTAGATTTGTCACAAGATTGGGCTCTGC

Example reaction

d2H2O	70uL
10x buffer	10uL
100mM MgSO4	0uL
2mM dNTP	10uL
Template 1(1ng/ uL)	1uL
Template 2(1ng/ uL)	1uL
Top Primer 25pmol/ uL	4uL
Bottom Primer 25 pmol/ uL	4uL
Vent Polymerase	0.5uL

PCR Assembly Product



FIG. 10B

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Strategy for Assembly of Chimeric 2D12.5
Heavy Chain
Step 3

Desired PCR Assembly Product



Restriction Digest PCR Product with Bgl II & Xba I



Ligate Restriction Digested PCR Product into pMTBipV5His
(S2 Cell Expression Vector, Propagated in XL-1 Blue E. Coli)

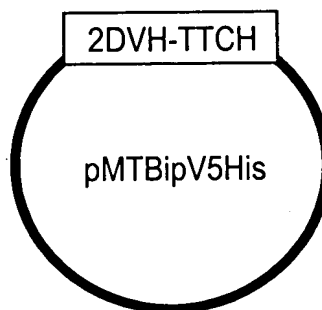


FIG. 10C

Step 1 - Mutation Methodology (PCR Reaction MT-VENT)

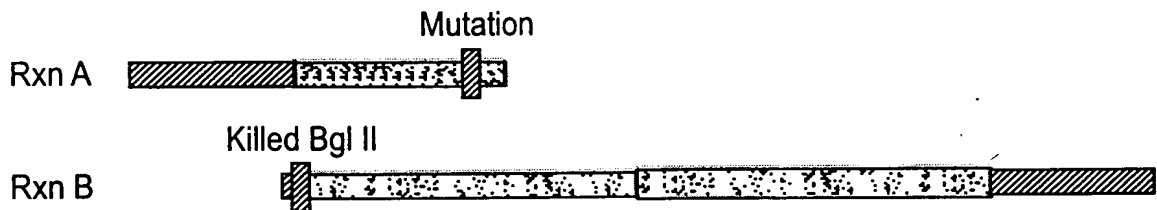


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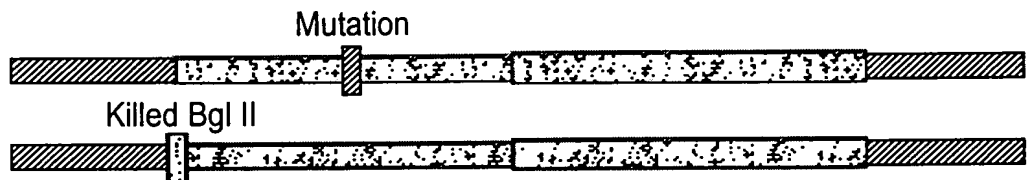
Step 2 - Mutation Methodology (PCR Reaction VHMUTTAQ)

2nd PCR Reaction (Mix Products of reaction A and B)

- 1) Extend
- 2) Amplify with outer primers (MTforward and BGHreverse)



2nd PCR Reaction Products (Mixture - 2 Products of equal size)



Restriction Digest PCR Product Mixture with BglII and Xba1

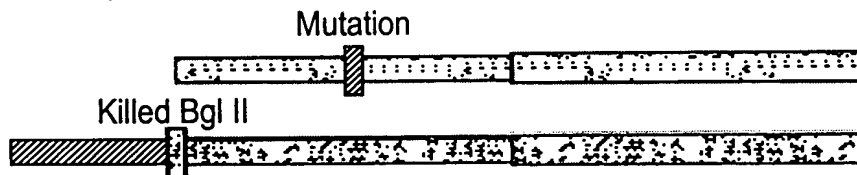


FIG. 10E

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Step 1 - PCR Reaction MT-VENT

PCR cycle - MT-VENT

1 - 95° C 10 min
2 - 94° C 1 min
3 - 50° C 1 min
4 - 75° C 2 min
5 - 24x to step 2
6 - 75° C 5 min
7 - 4° C 18 hr
8 - END
VENT added before step 2
Primers added before step 1

Example reaction

d2H2O	70ul
10x buffer	10uL
100mM MgSO4	0uL
2mM dNTP	10uL
Template (1ng/uL)	1uL
Top Primer 25pmol/uL	4uL
Bottom Primer 25pmol/uL	4uL
Vent Polymerase	0.5uL

Step 2 - PCR Reaction VHMUTTAQ

PCR cycle - VHMUTTAQ

1 - 95° C 10 min
2 - 94° C 1 min
3 - 68° C 1 min
4 - 72° C 2 min
5 - 4x to step 2
6 - 94° C 1 min
7 - 50° C 2 min
8 - 72° C 2 min
9 - 24x to step 6
10 - 72° C 5 min
11 - 4° C 18 hr
12 - END
Taq added before step 2
Primers added before step 6

Example reaction

d2H2O	61ul
10x buffer	10uL
25mM MgCl2	8uL
2mM dNTP	10uL
Template 1 (1ng/uL)	1uL
Template 2 (1ng/uL)	1uL
Top Primer 25pmol/uL	4uL
Bottom Primer 25pmol/uL	4uL
Taq Polymerase	0.5uL

FIG. 10F

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Strategy for Assembly of Chimeric 2D12.5
 Light Chain
 Step 1

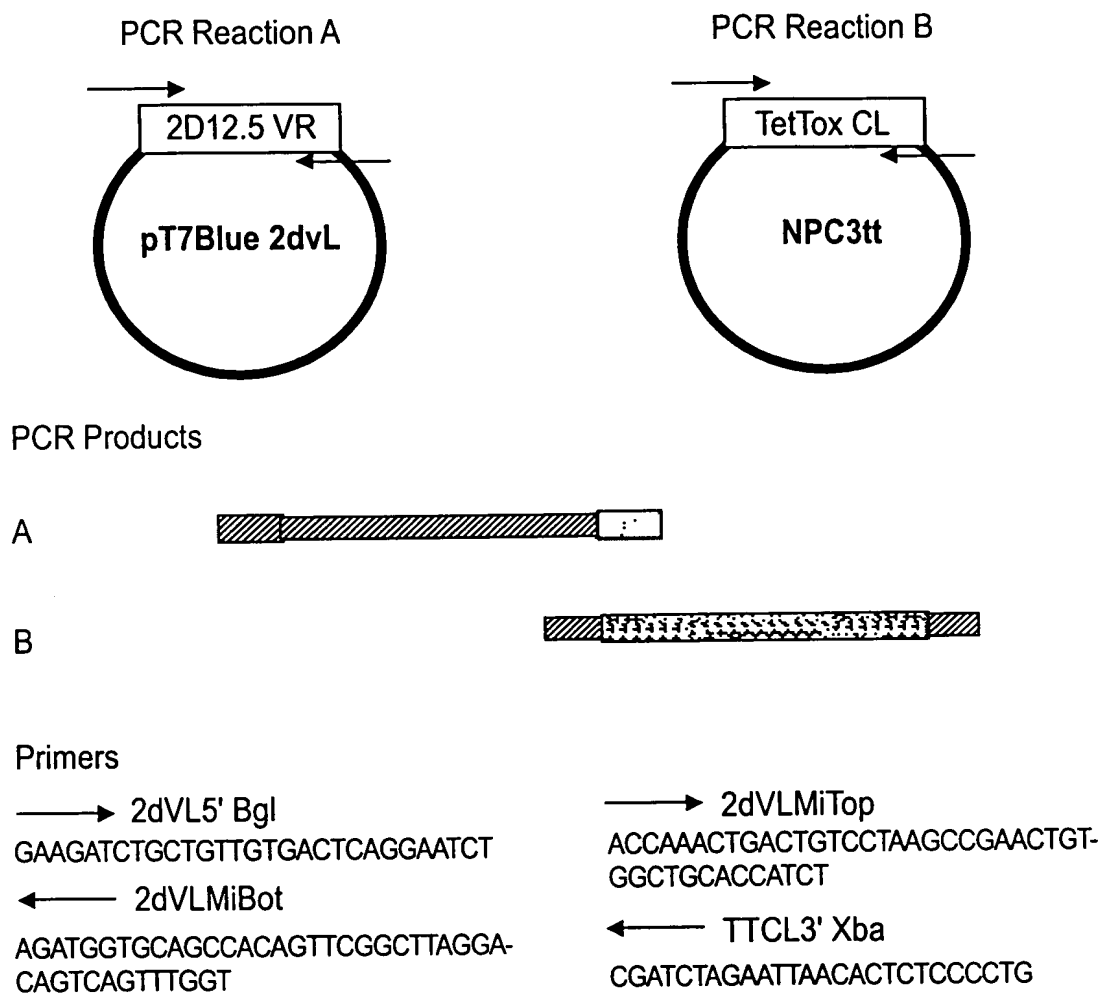
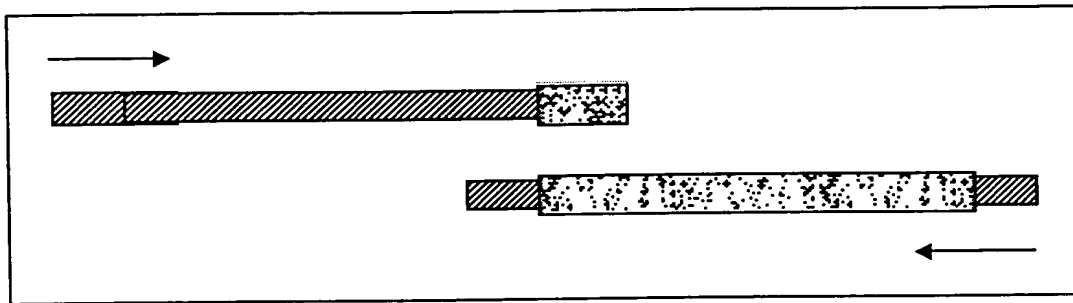


FIG. 11A

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Strategy for Assembly of Chimeric 2D12.5
Light Chain
Step 2

PCR Reaction



Primers

→ 2dVL5' Bgl
GAAGATCTGCTGTTGTGACTCAGGAATCT

← TTCL3' Xba
CGATCTAGAATTAACACTCTCCCCTG

PCR Assembly Product



Problem: There is an unwanted G48S mutation in the variable domain.

Solution: Repair with an S48G primer using the method of Ito. Also institute a mutation at N53C and repair point mutation with a single primer named S48G_N53C.

FIG. 11B

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Strategy for Assembly of Chimeric 2D12.5
Light Chain
Step 3

Desired PCR Assembly Product



Restriction Digest PCR Product with Bgl II & Xba I



Ligate Restriction Digested PCR Product into pMTBipV5His
(S2 Cell Expression Vector, Propagated in XL-1 Blue E. Coli)

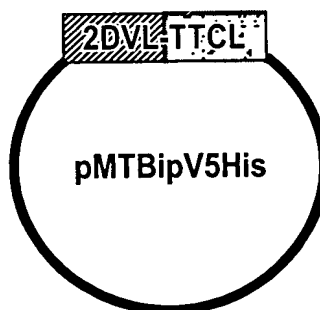
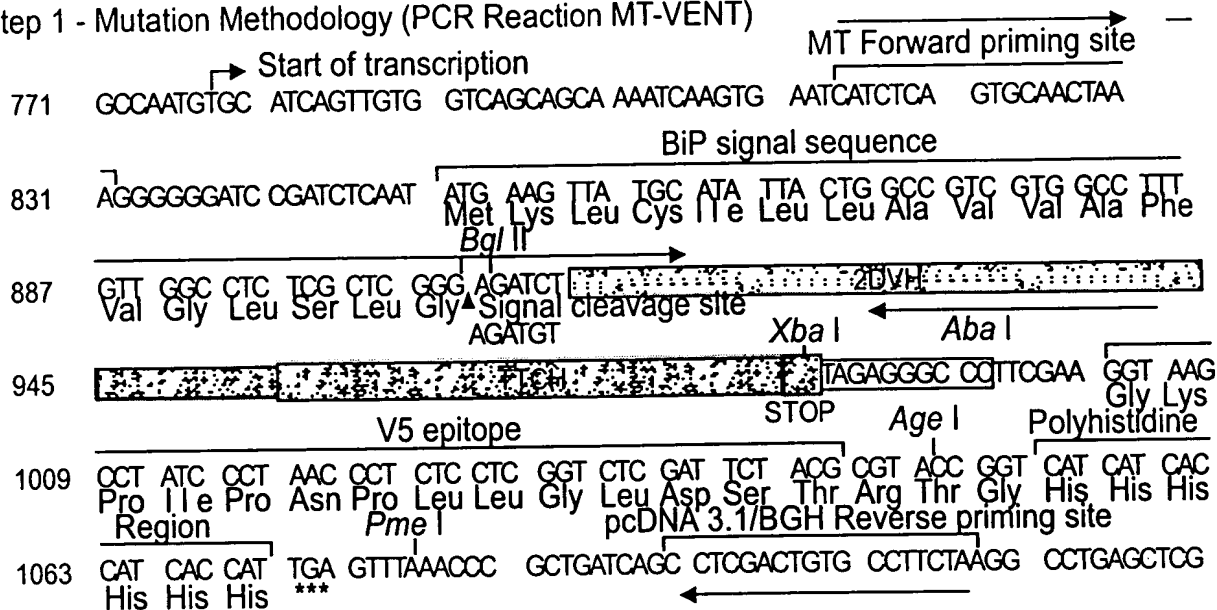


FIG. 11C

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Step 1 - Mutation Methodology (PCR Reaction MT-VENT)



1st Set of PCR Reactions Product A and B)

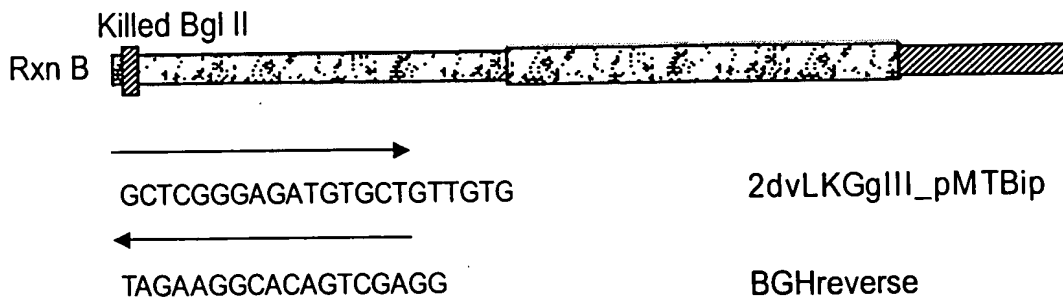
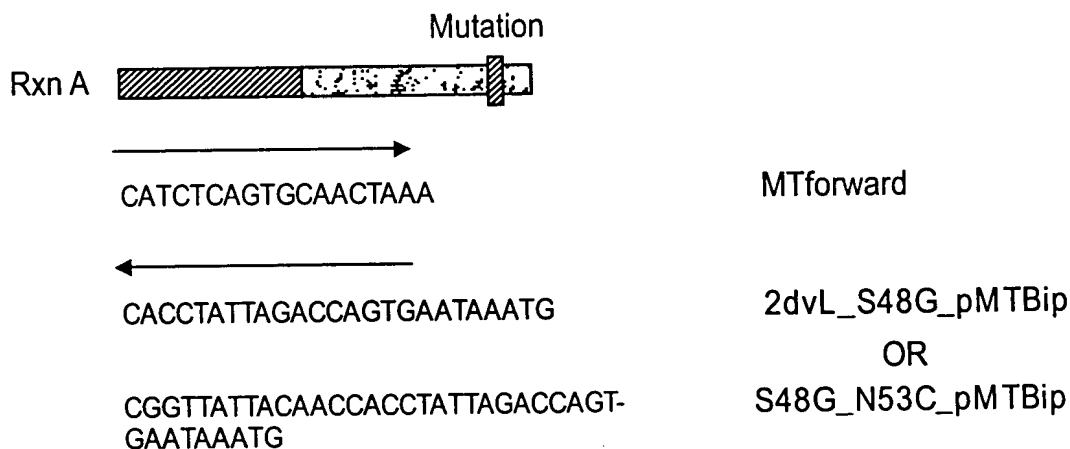


FIG. 11D

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Step 2 - Mutation Methodology (PCR Reaction VHMUTTAQ)

2nd PCR Reaction Mix Products of reaction B)

1) Extend

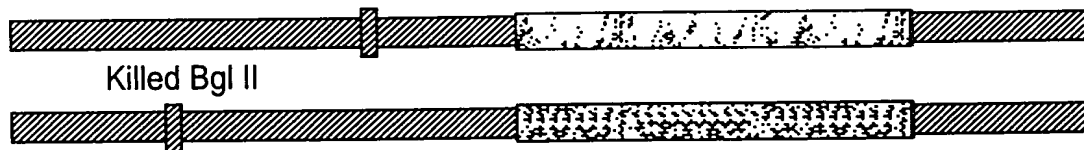
2) Amplify with outer primers (MTforward and BGHreverse)

Mutation



2nd PCR Reaction Products (Mixture - 2 Products of equal size)

Mutation



Restriction Digest PCR Product Mixture with BglII and Xba1

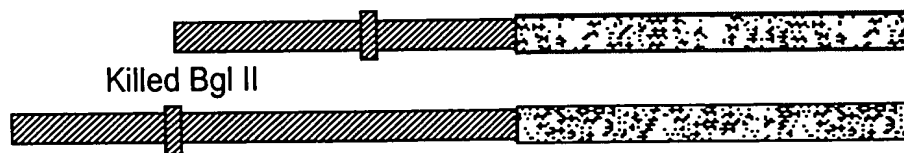
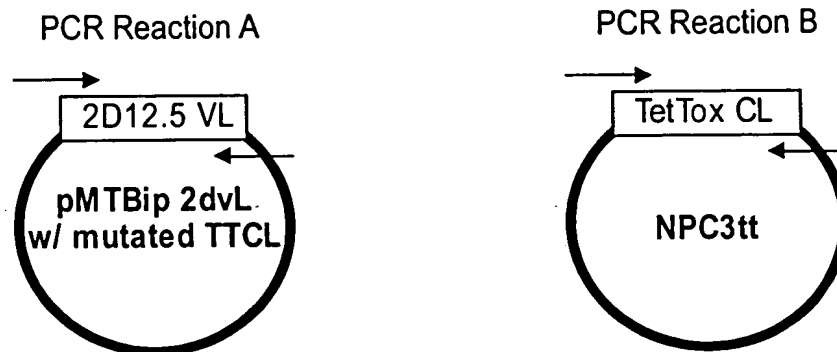


FIG. 11E

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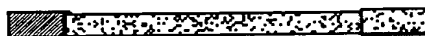
Strategy for Assembly of Chimeric 2D12.5 Light Chain

Step 4

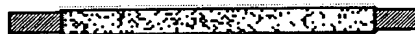


PCR Products

A



B



PCR cycle - 2DTTOver

- 1 - 94° C 10 min
 - 2 - 94° C 1 min
 - 3 - 62° C 1 min
 - 4 - 72° C 2 min
 - 5 - 25x to step 2
 - 6 - 72° C 5 min
 - 7 - 4° C 18 hr
 - 8 - END
- Taq added before step 2

Example reaction

d2H2O		63ul
10x buffer		10uL
25mM MgCl2	8uL	
2mM dNTP		10uL
Template 1 (1ng/ uL)	1uL	
Top Primer 25pmol/ uL		4uL
Bottom Primer 25 pmol/ uL	4uL	
Taq Polymerase		0.5uL

Primers

→ Mtforward
CATCTCAGTGCAACTAAA

← 2dVLMiBot
AGATGGTGCAGCCACAGTTTCGGCTTAGGA-
CAGTCAGTTTGGT

→ 2dVLMiTop
ACCAAACTGACTGTCCTAAGCCGAAC-
GTGGCTGCAACATCT

← TTCL3' Xba
CGATCTAGAATTAACACTCTCCCTG

FIG. 11F

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Assembled Vectors for Transfection in S2 Cells

Each of the following has been cotransfected
with the native light chain:

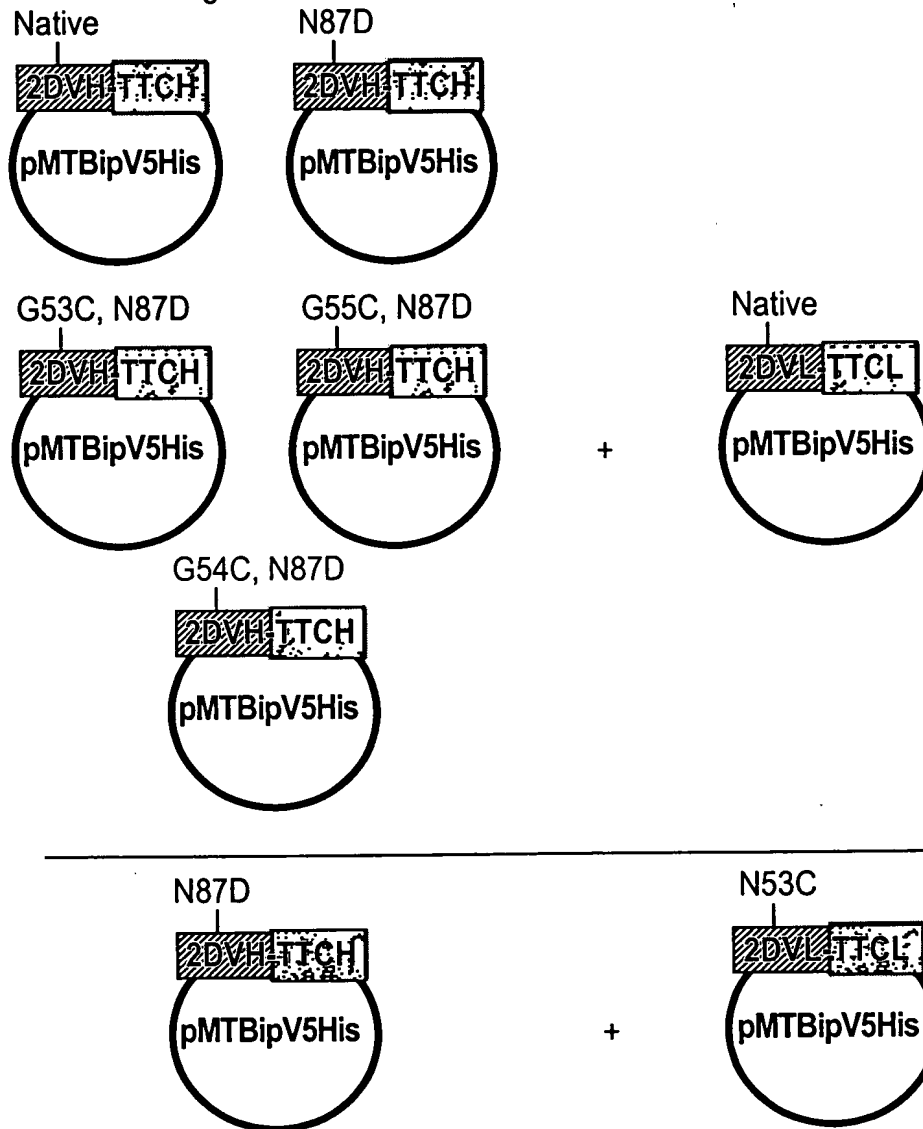


FIG. 11G

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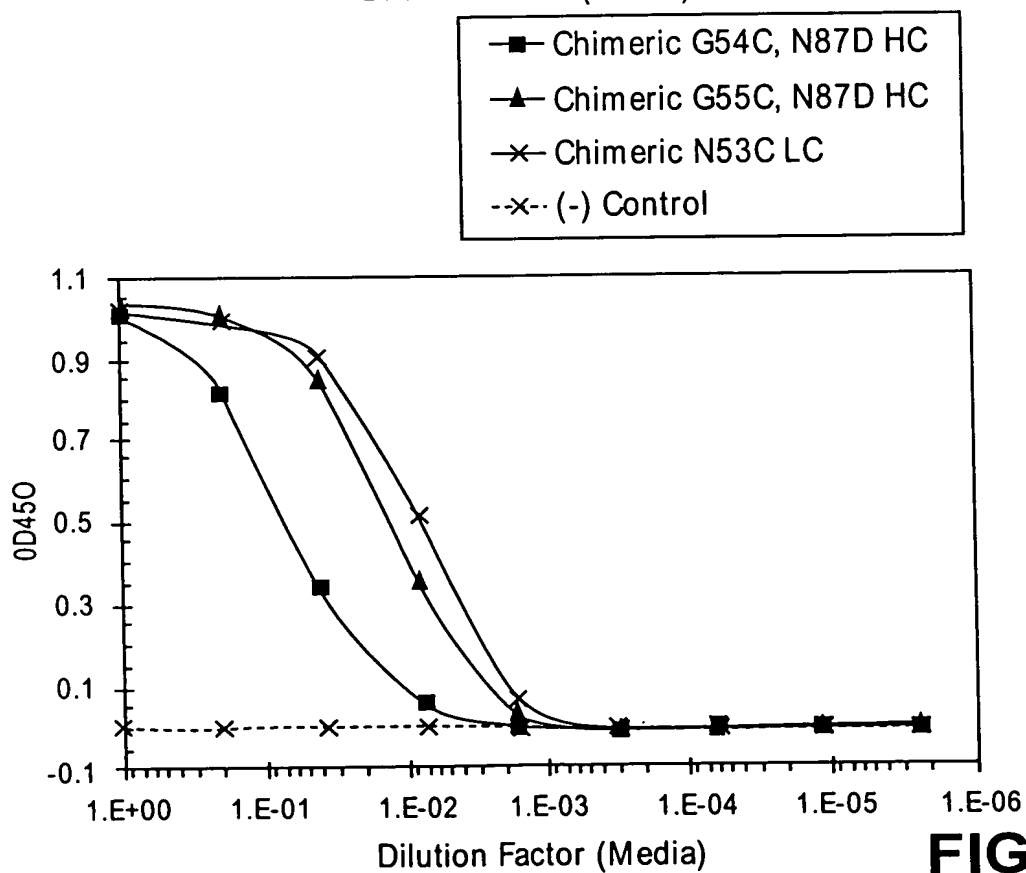
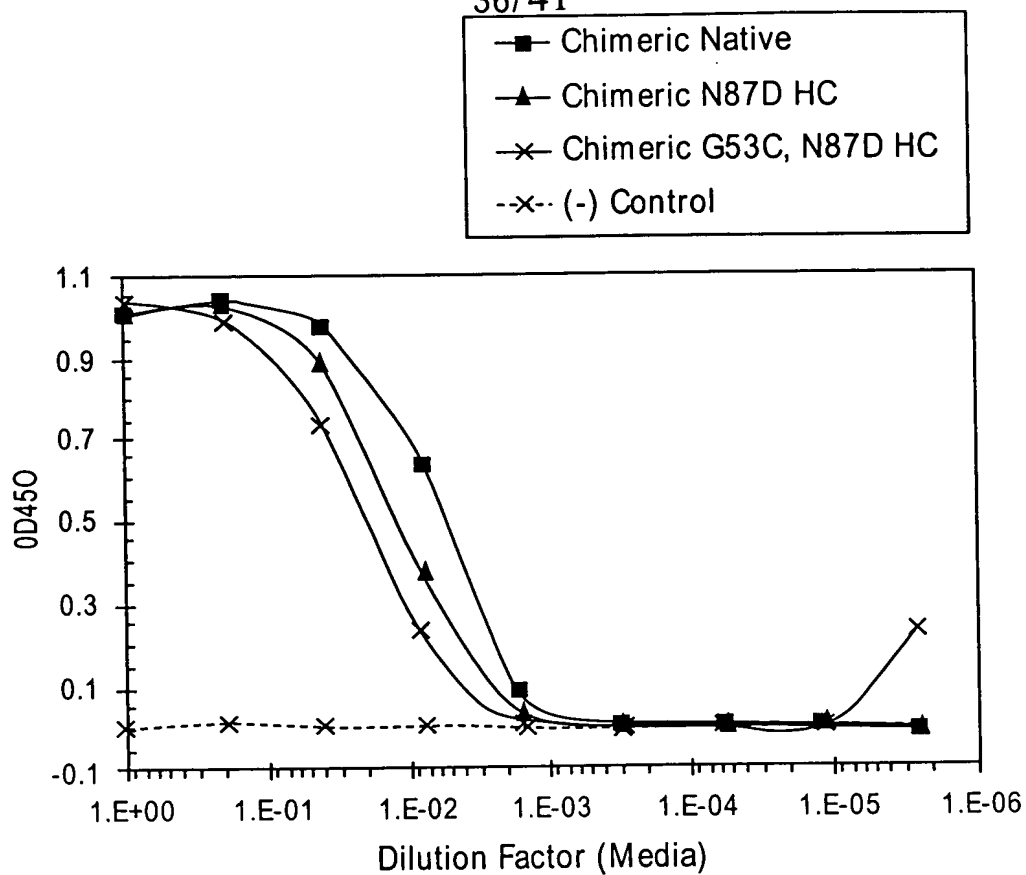


FIG. 12

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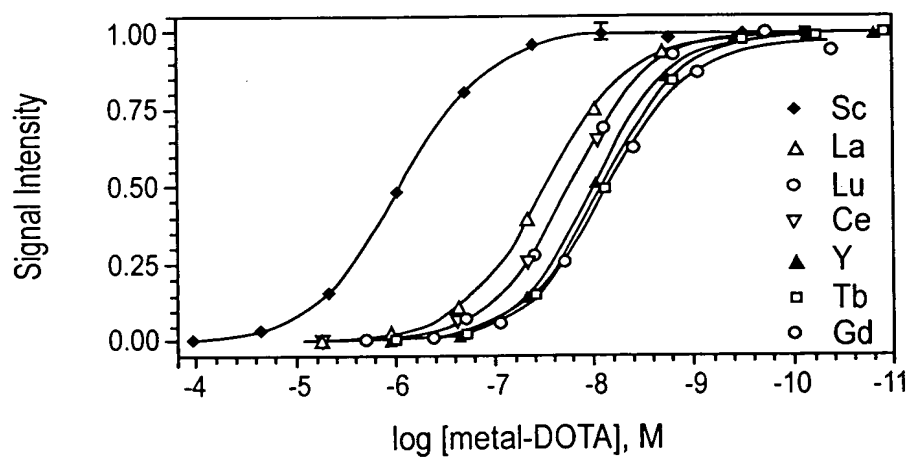


FIG. 13

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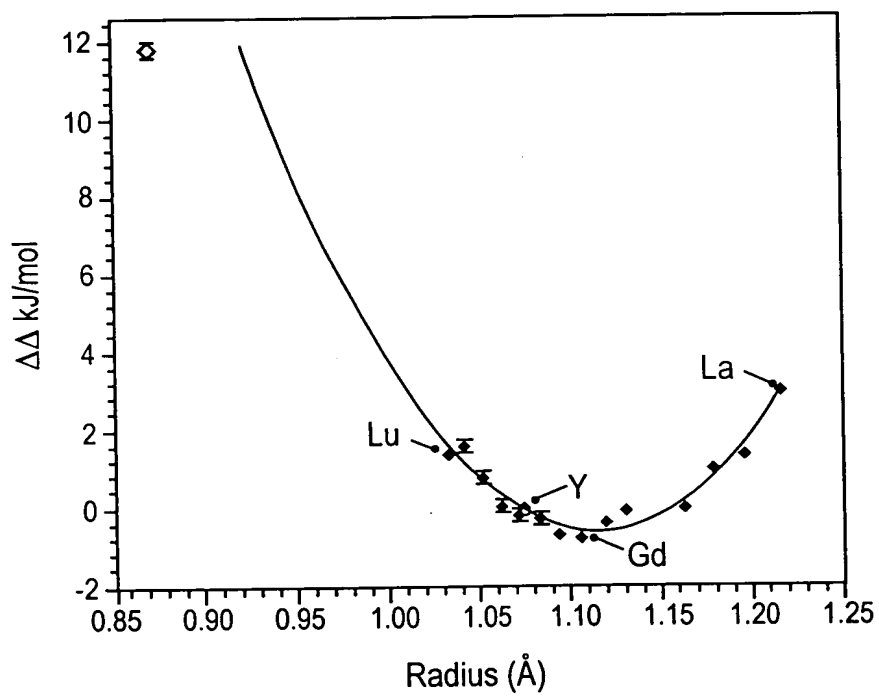


FIG. 14

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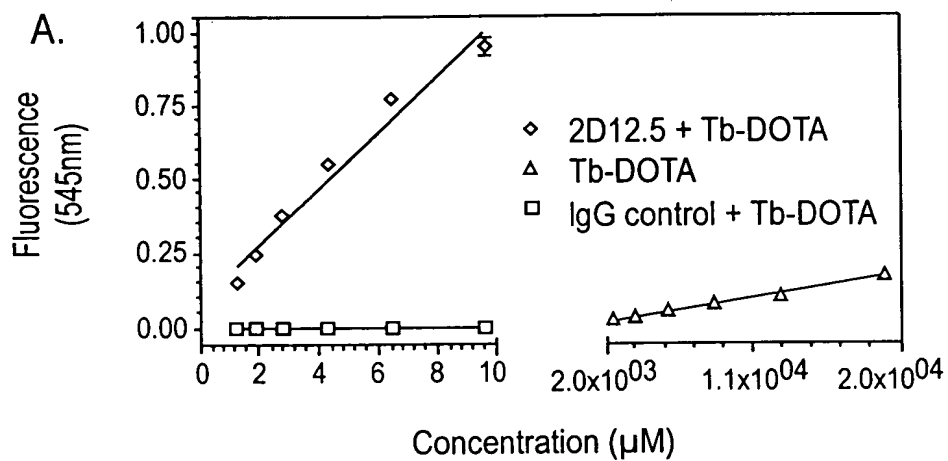


FIG. 15

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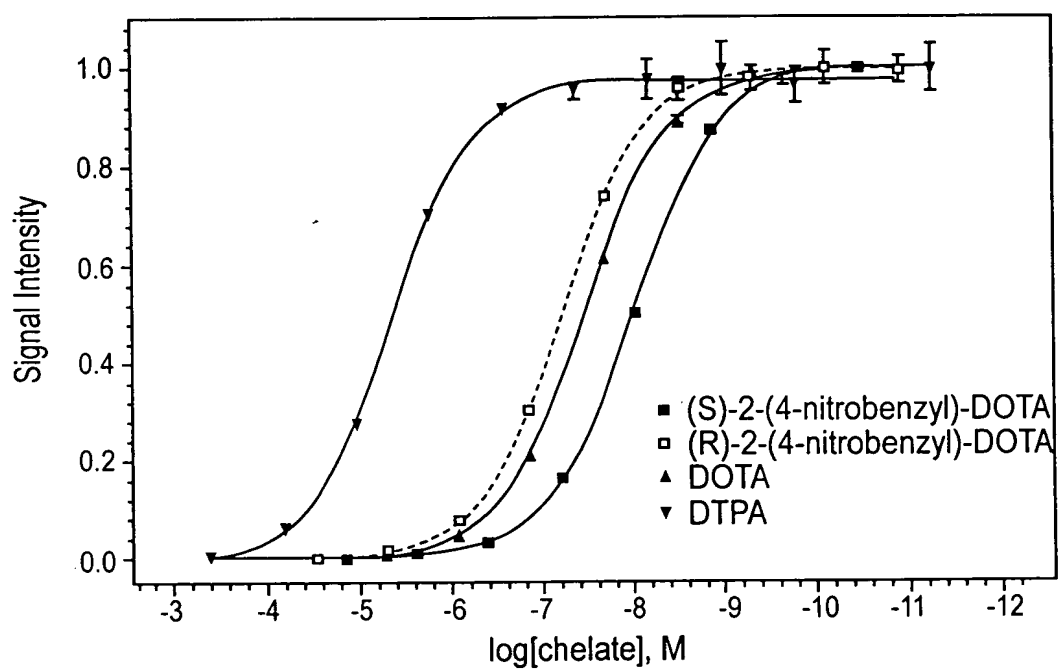


FIG. 16

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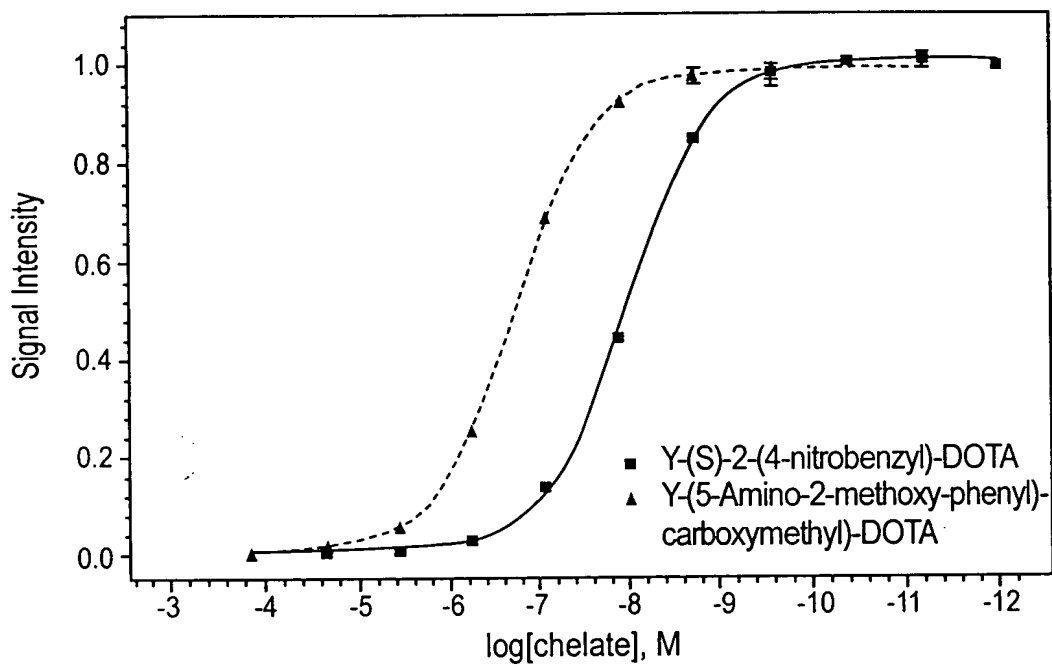


FIG. 17